GENETIC ANALYSIS OF ISOZYME LOCI IN TETRAPLOID POTATOES (*SOLANUM TUBEROSUM* L.)

J. M. MARTINEZ-ZAPATER AND JOSE L. OLIVER

Departamento de Genética, C-XV, Facultad de Ciencias, Universidad Autónoma de Madrid, Madrid-34, Spain

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ABSTRACT

The genetic control of eight isozyme loci revealed by starch gel electrophoresis was studied through the analysis of three progenies derived from four tetraploid cultivars of *Solanum tuberosum* (groups Andigena and Tuberosum). Duplicate gene expression was found in seven (*Got-A, Got-B, Pgd-C, Pgi-B, Pgm-A, Pgm-B* and *Pox-C*) isozyme loci. In another isozyme gene (*Adh-A*), the parental genotypes were not adequate to distinguish between a monogenic or a digenic model of genetic control. Tetrasomic inheritance was demonstrated in four (*Got-A, Got-B, Pgd-C* and *Pgi-B*) isozyme loci. In the remaining duplicate genes, the parental genotypes precluded discrimination between disomic or tetrasomic models. Tetrasomic segregations of the chromosomal type were generally found; however, the isozyme phenotypes shown by three descendants from selfing cv. Katahdin indicate the occurrence of chromatid segregations, although aneuploidy cannot be ruled out. Either autoploidy or amphidiploidy with lack of chromosome differentiation between the two diploid ancestors can account for the existence of tetrasomic inheritance in the common potato.

ISOZYME techniques have proved to be a very useful tool in biochemical genetic studies of both alloploid (ROOSE and GOTTLIEB 1976) and autoploid (QUIROS 1982, 1983) plant species; they allow the analysis of many genetic markers of codominant expression. Allelic isozymes (allozymes) can be used as markers of chromosomes or chromosome regions for the design of selection and breeding experiments. The use of allozymes as markers of loci in close association with genes for given traits will be crucial to the future success of plant breeding and genetic engineering (WHITT 1983). The elucidation of the genetic control of isozyme systems is also necessary for their use as markers for both varietal identification and phylogenetic analysis.

Cultivated potatoes belong to the series Tuberosa Rydb. of the genus Solanum. Differences exist in the number of species considered by distinct systematic classifications. Although HAWKES (1956a, 1978) recognizes eight species of cultivated potatoes, DODDS and PAXMAN (1962) consider only one species, *S. tuberosum* L., with five main groups: Stenotomum ($2x = 24$), Phureja ($2x = 24$), Chaucha ($3x = 36$), Andigena ($4x = 48$) and Tuberosum ($4x = 48$). The derivation of the potato cultivated in the Northern Hemisphere (*S. tuberosum* group Tuberosum) from the Sudamerican tetraploid potatoes is a well-

established fact, although some dispute remains about the group from which it was derived (Andigena from Perú and Northern Bolivia *vs.* Tuberosum from Chile) (see SWAMINATHAN and MAGOON 1961; HOWARD 1970; UGENT 1970; HAWKES 1978; GRUN 1979 for revisions). Different polyploidization mechanisms have been implicated for the origin of cultivated tetraploid potatoes: autoploidy from the diploid group Stenotomum (HAWKES 1956b), intervarietal autoploidy (STEBBINS 1957), segmental alloploidy (MATSUBAYASHI 1960) and amphidiploidy from Stenotomum and *S. vernei* (BRÜCHER 1964) or from Stenotomum and *S. sparsipilum* (HAWKES 1967; HOWARD 1973; but see WOODCOCK and HOWARD 1975). Because the frequency of multivalent associations at meiosis varies among different potato cultivars, cytogenetic data have not provided sufficient evidence to decide which of these mechanisms is involved (SWAMINATHAN and MAGOON 1961; HOWARD 1970). Tetrasomic ratios of inheritance have been established only in a few genes controlling both morphological characters and disease resistances; furthermore, some of the reported segregations can be also explained by monogenic models (SWAMINATHAN and MAGOON 1961; HOWARD 1970). More genes must be analyzed before any strong inference on the nature of ploidy can be drawn.

The basic problem with most of the traits genetically studied in the potato until now is the lack of equivalence between phenotype and genotype due to complications introduced by varying heritabilities, dominance, epistasis and pleiotropy. The study of isozyme genes avoids all of these problems, allowing the analysis of the complex segregations that can be expected in a polyploid.

We have undertaken an electrophoretic analysis of several enzymes in diploid and tetraploid groups of cultivated potatoes, as well as in some related wild species. The use of isozyme phenotypes for the identification of 67 Tuberosum varieties, including those of greatest agronomical interest in Europe and North America, has been previously reported (MARTINEZ-ZAPATER and OLIVER 1984). The phylogenetic relationships, as deduced from the analysis of gene frequencies, will be published elsewhere (OLIVER and MARTINEZ-ZAPATER 1984). We report here the inheritance analysis that we have carried out in four tetraploid cultivars at eight variable isozyme loci.

MATERIALS AND METHODS

Three progenies from four tetraploid cultivars of *S. tuberosum* were analyzed: cv. Katahdin selfed, cv. Turia selfed and Buesa ♀ × DTO-33♂.

The number of plants analyzed at each isozyme loci is shown in Tables 2–4. Katahdin, Turia and Buesa are typical Tuberosum cultivars, whereas the clone DTO-33 is an Andigena one. Seeds of the last two progenies and tubers of all these cultivars were provided by Estación de Mejora de la Patata, Vitoria, Spain. To obtain berries, cut stems with flowers were taken from field-grown plants and placed in jars of water. Plants were grown from seeds under uniform conditions in the greenhouse.

Three organs of the plant were analyzed: young leaves [phosphoglucose isomerase (PGI), phosphoglucomutase (PGM) and 6-phosphogluconate dehydrogenase (PGD)], tubers [alcohol dehydrogenase (ADH) and glutamate oxaloacetate transaminase (GOT)], and shoots [peroxidase (POX)]. In order to prevent browning, the enzyme extraction was accomplished by crushing the plant material in a buffered solution of several reducing agents (VALIZADEH 1977). For PGD and PGM enzymes, glycerol (10%) was added to the extraction buffer (ROOSE and GOTTLIEB 1980). The

TABLE 1

Allozyme relative mobilities at different isozyme systems in the potato cultivars analyzed in this study

Isozyme system	Allozymes			
	a	b	c	d
ADH-A		0.51	0.47	
GOT-A	0.46	0.40		
GOT-B			0.22	0.14
PGD-C	0.72	0.70		
PGI-B		0.37	0.33	
PGM-A	0.52	0.50		
PGM-B		0.40	0.34	
POX-C	0.54		0.50	

enzyme extracts were adsorbed directly onto paper wicks and subjected to horizontal starch gel electrophoresis, with LiOH/borate (pH 8.1) electrode buffer and Tris/citrate (pH 8.3) gel buffer (SELANDER *et al.* 1971). For PGD we added EDTA 0.4M to gel and electrode buffers. Samples of cv. Desiree were included in all of the slab gels as internal markers to determine the electrophoretic mobilities of the different allozyme bands.

Different gel slices were assayed for six consistently scorable enzymes: ADH [EC 1.1.1.1 (PASTEUR 1973)], GOT [EC 2.6.1.1 (GOTTLIEB 1973)], PGI [EC 5.3.1.9 (BREWER 1970)], PGM [EC 2.7.5.1 (BREWER 1970)]; POX [EC 1.11.1.7 (SHAW and PRASAD 1970) with the pH modified at 4.5 according to RICK, ZOBEL and FOBES (1974)], PGD [EC 1.1.1.43 (BREWER 1970)].

Isozyme system, locus and allele letter names were assigned following an electrophoretic survey of different diploid and tetraploid groups of *S. tuberosum* and of two diploid wild spcies, *S. sparsipilum* and *S. pinnatisectum* (OLIVER and MARTINEZ-ZAPATER 1984). Isozyme system names begin with a capitalized abbreviation for an already recognized enzyme name (*e.g.*, GOT); the isozyme system with the most anodal migration was designated A, the next B and so forth (*e.g.*, GOT-A, GOT-B). In order to distinguish the isozyme locus from the protein it encoded, the corresponding abbreviations (*e.g.*, *Got-A*, *Got-B*) is italicized. For each isozyme locus, the allele with the greatest relative mobility was called *a*, and then *b*, *c*, *d*, etc.

RESULTS

Electrophoretic patterns from 11 different tissues and organs (MARTINEZ-ZAPATER 1983; MARTINEZ-ZAPATER and OLIVER 1984; OLIVER and MARTINEZ-ZAPATER 1984) provided an estimate of the number of isozyme systems for each enzyme in Tuberosum cultivars. Eight isozyme systems were selected for study through progeny analysis. The relative mobilities of the alleles found in the cultivars analyzed here are shown in Table 1. In addition, other electrophoretic bands, whose presence cannot be genetically explained, were detected in PGD and PGI enzymes. These were attributed to epigenetic modifications. Those detected at PGI-B were similar to those reported by STAUB *et al.* (1982).

The four tetraploid cultivars analyzed here were variable for some of the eight isozyme systems. Different classes of putative heterozygotes with reciprocal asymmetric banding intensities were observed. Let us consider, for example, a dimeric isozyme system, ADH-B, for which five electrophoretic phenotypes were found (Figure 1). In addition to the normal tribanded hetero-

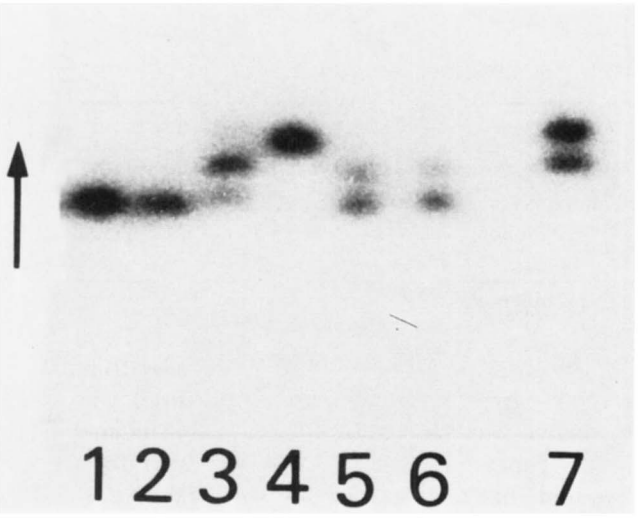

FIGURE 1.—Phenotypes for a dimeric potato isozyme (ADH-B) in the tetraploid group Andigena of *S. tuberosum*. Note the change in the relative staining intensity of the heterodimeric and homodimeric bands in symmetric triple-banded phenotypes (lane 3) if compared with the asymmetric ones (lanes 5, 6 and 7). Thus, in the symmetric heterozygote the heterodimer (middle band) shows the highest intensity. However, in the asymmetric heterozygotes one of the homodimers (slow band in lanes 5 and 6, fast band in lane 7) is the most stained. The following genotypes can be assigned: 1 and 2, *cccc*; 3, *aacc*; 4, *aaaa*; 5 and 6, *accc*; 7, *aaac*.

zygote (lane 3), two other tribanded phenotypes with asymmetrical banding intensities were observed: the first one has the slow homodimeric band more intensely stained (lanes 5 and 6), whereas the second one represents the reciprocal situation with the fast homodimeric band more intensely stained (lane 7). Slow (lanes 1 and 2) and fast (lane 4) homozygotes were also observed. Since groups Andigena and Tuberosum are recently originated tetraploids (UGENT, POZORSKY and POZORSKY 1983), duplicate gene expression must be expected (OLIVER *et al.*, 1983). In plants, gene dosage can result in an increase in the amount of gene product and, consequently, the relative intensity of individual electrophoretic bands (CARLSON 1972; DEMAGGIO and LAMBRUKOS 1974; ROOSE and GOTTLIEB 1980). Thus, the best explanation for these phenotypes is that they are due to gene dosage effects (see, for example, ALLENDORF, UTTER and MAY 1975). That the relative band intensity is a result of gene dosage in the potato is further supported by the observation that triploid plants of varieties Negra and Chaucha Colorada always show an asymmetric phenotype at all those isozyme loci for which they are heterozygous. In tetraploid potatoes, reciprocal asymmetric banding intensities can be readily detected on the gels, and they were observed at each one of the eight variable isozyme systems; thus, a genotype can be deduced for each electrophoretic phenotype (Figure 1).

For each isozyme locus, we analyzed those progenies in which either one or both parents were putative heterozygotes. Consideration of gene dosage effects was not necessary to discriminate between the different genetic models. We

TABLE 2

Segregation of b *and* c *alleles at* Adh-A *isozyme locus*

Isozyme locus	Phenotypes				χ^2	*P*
	Parents		Offspring			
	BUESA × DTO-33		bbbb	bbbc		
Adh-A	(bbbc × bbbb)	Obs. no.	107	90		
		Exp. ratio	1	1	1.47	0.23

Exp., expected; Obs, observed.

tested first the hypothesis of a monogenic control. If this possibility was ruled out by χ^2 tests, the hypothesis of a digenic model with either disomic or tetrasomic inheritance was then tested. Once a particular mode of inheritance was demonstrated, gene dosage effects were taken into account to again test the particular model that follows each isozyme gene. For the sake of brevity, we only present here the tests that consider gene dosage effects (Tables 2–4). For the analysis of *Pgd-C* in the cross Buesa × DTO-33 (Table 4), all of the tribanded phenotypes were pooled together; the corresponding gels were not well enough resolved to score gene dosage effects, and the analyses could not be repeated due to the destruction of the plants for the first analysis.

The results obtained for *Adh-A* are shown in Table 2. Buesa shows a tribanded electrophoretic phenotype with the fast homodimeric band more intensely stained, whereas DTO-33 shows only one band, corresponding to the fast homodimer. The progeny shows these two phenotypes in a 1:1 ratio, indicating that variation observed for this isozyme system was under genetic control. However, a 1:1 ratio would be expected whether control is monogenic (parental genotypes: *b/c* × *b/b*) or digenic (disomic: *b/b b/c* × *b/b b/b*; or tetrasomic: *bbbc* × *bbbb*), precluding discrimination between these genetic models.

For the remaining seven loci, evidence was obtained that supports duplicate gene expression (Tables 3 and 4). The observed segregations for *Pgm-A*, *Pgm-B* and *Pox-C* fit well with expected ratios according to a digenic model (Table 3). Because of the triplex constitution of one or both parents for these isozyme loci, we cannot distinguish between disomic or tetrasomic inheritance. However, segregations for *Got-A*, *Got-B*, *Pgd-C* and *Pgi-B* (Table 4) allowed us to discriminate between both patterns of digenic inheritance, due to the duplex constitutions of one or both parents in at least one of the progenies analyzed. The observed segregations fit well with a tetrasomic model in which chromosomal segregation occurs.

In the progeny from selfing cv. Katahdin we found three individuals with unexpected phenotypes, if tetrasomic inheritance with only chromosome segregation was assumed. For *Got-A* and *Pgi-B* we found individuals with asymmetrical banding intensities, the band corresponding to the homodimers bb being most intensely stained; for *Pgd-C* another asymmetrical banding appeared, the band corresponding to the homodimer aa being most intensely stained.

TABLE 3

Segregations analyzed for Pgm-A, Pgm-B *and* Pox-C *isozyme loci*

Isozyme locus	Parents	Phenotypes: Offspring Obs. no. (Exp. ratio)			χ^2	*P*
		aabb	abbb	bbbb		
Pgm-A	KATAHDIN S (abbb × abbb)	22 (1)	49 (2)	22 (1)	0.27	0.87
		bbbb	bbbc	bbcc		
Pgm-B	KATAHDIN S (bbbc × bbbc)	26 (1)	50 (2)	22 (1)	0.37	0.83
		bbbb	bbbc			
	BUESA × DTO-33 (bbbc × bbbb)	90 (1)	111 (1)		2.19	0.15
		aaaa	aaac	aacc		
Pox-C	KATAHDIN S (aaac × aaac)	20 (1)	30 (2)	12 (1)	2.13	0.35

Expected (Exp.) ratios were those of a digenic model. S indicates selfing; Obs., observed.

DISCUSSION

In the analysis that we have carried out on tetraploid potatoes, duplicate gene expression has been demonstrated for seven of eight variable isozyme loci (Tables 3 and 4). Only for *Adh-A* were the parental genotypes not adequate to distinguish between a monogenic or a digenic model of genetic control (Table 2). However, the reciprocal asymmetric banding intensities observed in Buesa and other tetraploid cultivars for this locus (MARTINEZ-ZAPATER and OLIVER 1984) indicate that it is duplicated as well. Thus, a digenic control exists for all of the isozyme systems we have studied. These results suggest that this would be the general pattern of inheritance in tetraploid potatoes.

Tetrasomic inheritance has been demonstrated in four of seven duplicate isozyme loci (Table 4). In three other duplicate genes, the parental genotypes precluded discrimination between disomic or tetrasomic models (Table 3). Tetrasomic inheritance has been previously reported in both groups of tetraploid potatoes, Tuberosum and Andigena, for genes controlling morphological characters, as well as for those conferring disease resistances (SWAMINATHAN and MAGOON 1961; HOWARD 1970).

Generally, for tetrasomic isozyme loci we found segregations to be of the chromosomal type (Table 4). However, in the progeny resulting from selfing cv. Katahdin one individual for each one of three isozyme loci (*Got-A*, *Pgd-C* and *Pgi-B*) showed electrophoretic phenotypes that could be explained by chromatid segregations; the corresponding genotypes would be *abbb* for *Got-A*, *aaab* for *Pgd-C* and *bbbc* for *Pgi-B*. Chromatid segregation has been previously observed in group Tuberosum for genes showing tetrasomic inheritance (SWAMINATHAN and MAGOON 1961; HOWARD 1970). However, similar electropho-

TABLE 4

Segregations analyzed for Got-A, Got-B, Pgd-B *and* Pgi-B *isozyme loci*

Isozyme locus	Parents	Phenotypes of Offspring						χ^2	*P*
			aaaa	aaab	aabb				
Got-A	KATAHDIN S (aaab × aaab)	Obs. no.	23	40	23				
		Exp. ratio							
		Disomic or tetrasomic	1	2	1			0.42	0.81
			aaaa	aaab	aabb	abbb			
	BUESA × DTO-33 (aabb × aaab)	Obs. no.	9	82	91	14			
		Exp. ratio							
		Disomic (aa/bb × aa/ab)	1	3	3	1		19.46	<0.001
		Tetrasomic	1	5	5	1		4.67	0.20
			cccd	ccdd	cddd	dddd			
Got-B	BUESA × DTO-33 (ccdd × cddd)	Obs. no.	16	86	80	15			
		Exp. ratio							
		Disomic (cd/cd × cd/dd)	1	3	3	1		9.29	0.03
		Tetrasomic	1	5	5	1		0.37	0.94
			aabb	abbb	bbbb				
Pgd-C	KATAHDIN S (abbb × abbb)	Obs. no.	12	22	7				
		Exp. ratio							
		Disomic or tetrasomic	1	2	1			1.44	0.49
			aaaa	aaab	aabb	abbb	bbbb		
	BUESA × DTO-33 (aabb × aabb)	Obs. no.	5		193		5		
		Exp. ratio							
		Disomic (aa/bb × aa/bb)	1		14		1	10.67	<0.01
		Tetrasomic	1		34		1	0.14	0.93

TABLE 4—*Continued*

			bbcc	bccc	cccc				
Pgi-B	KATAHDIN S	Obs. no.	25	51	23				
	(bccc × bccc)	Exp. ratio							
		Disomic or tetrasomic	1	2	1			0.17	0.92
			bbbb	bbbc	bbcc	bccc	cccc		
	TURIA S	Obs. no.	2	16	43	19	0		
	(bbcc × bbcc)	Exp. ratio							
		Disomic (bb/cc × bb/cc)	1	4	6	4	1	13.28	0.01
		Tetrasomic	1	8	18	8	1	2.77	0.60

Only the most probable disomic constitution of the parents, according to the distribution of phenotypic classes observed in the progeny, were assumed in calculating expected disomic values. S indicates selfing; Exp., expected; obs., observed.

retic phenotypes would also result if these individuals originated from aneuploid gametes (CATCHESIDE 1959); then, the corresponding genotypes would be *abb* for *Got-A*, *aab* for *Pgd-C* and *bbc* for *Pgi-B*. Trivalents and univalents, which have been observed at meiosis of *S. tuberosum*, could lead to the formation of aneuploid gametes (HOWARD 1970).

We have observed tetrasomic inheritance at one or more isozyme loci in two Tuberosum cultivars (Buesa and Turia) as well as in an Andigena cultivar (DTO-33). The existence of tetrasomic inheritance in tetraploid potatoes reveals that lack of preferential pairing of chromosomes exists, a conclusion that can be also proposed from the observation of multivalent configurations at meiosis (SWAMINATHAN and MAGOON 1961; HOWARD 1970). This may be due to an autoploid origin of *S. tuberosum* (HAWKES 1956b) or, if an amphidiploid origin is assumed (HAWKES 1967), to little chromosome differentiation between both diploid ancestors. In most isozyme loci, the alleles of group Andigena were a combination of those found in both group Stenotomum and the diploid weed *S. sparsipilum* (OLIVER and MARTINEZ-ZAPATER 1984), suggesting an amphidiploid origin of group Andigena from those two diploid taxa. However, since Andigena was more related to Stenotomum (NEI unbiased genetic distance D = 0.052) than to *S. sparsipilum* (D = 0.241), the autoploidization of Stenotomum individuals and the subsequent hybridization with group Andigena may also occur. Therefore, models based on amphidiploidy, autoploidy and/or hybridization of the resulting tetraploids seem to be compatible with molecular data. Although this does not mean that all of these processes are necessarily involved, it clearly agrees with the proposal of UGENT (1970) on a multiple origin for cultivated potatoes (OLIVER and MARTINEZ-ZAPATER 1984). Thus, either autoploidy or amphidiploidy with lack of chromosome differentiation between the putative ancestors (Stenotomum and *S. sparsipilum*) (HAWKES 1978) can account for the existence of tetrasomic inheritance in the common potato.

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